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Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae†

RUNNING TITLE: Rearing substrate effects on performance and nutritional composition of black soldier fly

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ABSTRACT

BACKGROUND: Wastes can be used as rearing substrate by black soldier fly (BSF) larvae, the latter being exploitable as protein source in animal feed. This research aimed to assess the influence of four rearing substrates [Trial 1 (organic wastes): a mixture of vegetable and fruit (VEGFRU) *vs* a mixture of fruits only (FRU); Trial 2 (agro-industrial by-products): brewery (BRE) *vs* winery (WIN) by-products] on BSF larvae development, waste reduction efficiency, and nutritional composition.

RESULTS: If respectively compared to FRU and WIN, VEGFRU and BRE larvae needed less time to reach the prepupae stage (22.0, 22.2, 20.2 and 8.0 days of trial, respectively) and had higher protein content (229.7, 257.3, 312.9 and 395.7 g kg^{-1} DM). The waste reduction index ranged from 2.4 (WIN) to 5.3 g d^{-1} (BRE). BRE larvae showed the lowest saturated and the highest polyunsaturated fatty acids proportions (612.4 and 260.1 g kg^{-1} total fatty acids, respectively).

CONCLUSION: Vegetable and fruit wastes and winery by-products can be used as rearing substrates for BSF larvae mass production. Brewery by-products led to very promising larvae performances and nutritional composition. However, given BRE limited availability, low BRE dietary inclusion levels could be used with the purpose of increasing larvae performances.

Keywords: organic waste, agro-industrial by-product, *Hermetia illucens*, animal feed, crude protein, fatty acid profile

INTRODUCTION

The world population is estimated around 7.3 billion, with a growth rate of about 83 million per year. This increase will generate an increment of food demand with a consequent rise in waste and by-products production.[1] Urgent and innovative solutions are needed for the management of the waste streams (WS) that nowadays are estimated around 1.3 billion and 100 million tons per year in the world and in the European Union, respectively.[1,2] Furthermore, the EC Directive No 2008/98 unequivocally establishes the order of priority in the choice of WS treatment, the first being their reuse and the last their landfill disposal.

Some WS could be valorized through the recovery of the residual bio-elements they contain, with a cost reduction both for the industry (disposal cost) and the environment (pollution).[3] The use of insects in the bioconversion of WS constitutes a new approach and an interesting example of sustainable circular economy. This bioconversion can generate new elements such as proteins and lipids for animal feeds,[4,5,6,7] biodiesel,[8] high value products as chitin[9] or anti-microbial peptides.[10]

Processed proteins from seven insect species have recently been approved for aquafeed by the EC Regulation No 2017/893, which also lists the licensed rearing substrates. Among authorized species, black soldier fly (BSF; Diptera: Stratiomydae) is one of the most promising and researches recently aimed to increase knowledge on optimal rearing substrates for larvae and prepupae. In this respect, BSF has shown great flexibility as it can be used to reduce volume and add value to various wastes.[8,11,12] The available literature has highlighted that BSF life cycle and nutritional composition are noticeably influenced by the rearing substrate,[13,14] with the crude protein (CP) content of the larvae ranging from about 317 to 630 g kg^{-1} dry matter (DM).[7,15,16]

In 2014, around 90 million tons of slaughter and vegetable WS were produced in Europe (EUROSTAT(http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=env_wasgen&lang=en)). Considering the Italian context, 54% of the total production of waste

and agro-industrial by-products is generated by the manufacturing of vegetable products.[17] About 1.5 million tons of winery by-products and 406 tons of brewery by-products are produced every year (EUROSTAT(http://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language=en&pcode=tag00034&plugin=1; EU Report (https://www.brewersofeurope.org/site/media-centre/index.php?doc_id=905&class_id=31&detail=true)).[18]

The aim of this research was to evaluate the effects of organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector as rearing substrates for BSF larvae on their development, waste reduction efficiency and chemical composition.

MATERIAL AND METHODS

Two trials were carried out at the Experimental Facility of the Department of Agricultural, Forest and Food Sciences (DISAFA; University of Torino, Torino, Italy).

Rearing substrates

In Trial 1, two organic wastes were compared:

- Vegetable-fruit waste (VEGFRU) obtained from a street market (Torino, Italy) and containing a mixture of vegetables and fruits (celery 43.4%, oranges 28.9% and peppers 27.7%);
- Fruit waste (FRU) obtained from a fruit market (Torino, Italy) and containing fruits only (apples 47.8%, oranges 15.5%, apple leftovers 13.8%, strawberries 7.1%, mandarins 4.8%, pears 4.1%, kiwis 3.4%, bananas 1.9% and lemons 1.6%).

In Trial 2, two agro-industrial by-products were used:

- Winery by-product (WIN) obtained during the wine making process, before the alcohol extraction, from a private distillery (Distilleria Santa Teresa dei Fratelli Marolo S.R.L., Alba (CN), Italy) and containing grape seeds, pulp, skins, stems and leaves;

- Brewery by-product (BRE) obtained during beer production (IFN 5-00-517 Barley brewers grains wet) from a private brewery ("Birrificio dei Santi", Castelnuovo Don Bosco (AT), Italy).

Each substrate was ground with a 3 mm die meat mincer (FTS136; Fama Industrie S.r.l., Rimini, Italy) and carefully mixed.

A sample of each substrate was freeze-dried and frozen at -80°C for further chemical analysis, while the remaining was stored at -20°C until it was fed to the larvae.

BSF eggs

BSF eggs laid on corrugated cardboards for less than 24 h, were purchased from a private company (CIMI S.r.l., Cervasca (CN), Italy). The cardboards with the eggs were immediately transported to the DISAFA Experimental Facility. The cardboards were put onto plastic boxes (25cm × 33cm × 12cm) which contained whole rye thoroughly mixed with water (60% moisture) as rearing substrate for the newborn larvae. The plastic boxes were placed into climatic chambers under controlled environmental conditions (T: 27±0.5°C; RH: 70±5%; 24:0 L:D photoperiod). The eggs hatched approximately three days after oviposition.

Experimental design and calculations

Larvae development and waste reduction efficiency

Six-day-old larvae were used in both trials. In each trial, for the evaluation of larvae development (weight and length) and waste reduction efficiency, six replicates of 100 larvae were weighed (KERN PLE-N v. 2.2; KERN & Sohn GmbH, Balingen-Frommern, Germany; d: 0.001) and assigned to each rearing substrate. The method reported by Harnden and Tomberlin[19] was used to count the larvae. For each replicate, the larvae were placed into plastic containers (10cm × 17.5cm × 7cm), directly on the rearing substrate (100 g per replicate). The containers were covered with a perforated cap with a black nylon grid and placed in a climatic

chamber under controlled environmental conditions (T: 27±0.5°C; RH: 70±5%; 24:0 L:D photoperiod).

Each replicate was monitored daily to control the quantity of available feed. If needed, as reported by Harnden and Tomberlin,[19] 50 g of substrate per replicate was added in all replicates at the same time.

To avoid the effect of handling on the considered dependent variables,[13] weight and length data were collected every four days until the appearance of the first prepupae, thereafter every day for the relative substrate. Thirty larvae were randomly sampled for three consecutive times from each container to measure weight and length. As measurement was not destructive, the larvae were re-introduced into the containers between two consecutive samplings. The sampled larvae were individually cleaned, dried with a paper towel and weighed, and photographed orthogonally (Lumix G1; Panasonic Corporation, Kadoma, Osaka, Japan) with a metric scale (mm). The images were analyzed with ImageJ software package (v. 1.50b) to record larvae length (i.e., from mouthpart to the bottom of the last abdominal segment).

For each container, weight and length data collection ended when 30% of the larvae reached the prepupae stage. The prepupae were removed from the containers. The remaining 70% of the larvae were hand-counted, washed, dried with a paper towel and individually weighed and photographed. The total final biomass (larvae + prepupae) and the residual rearing substrate were also weighed. The following parameters were then calculated:

– larvae mortality (LM)

LM = [initial number of larvae - (final number of larvae + number of prepupae)] / initial number of larvae * 100;

- growth rate (GR),[20] readapted for this research substituting prepupa body weight (g) with larva body weight (g)

GR = (larva average final body weight (g) - larva initial body weight (g)) / days of trial (d);

- substrate reduction (SR)[21]

SR = [(distributed substrate (g) - residual substrate (g)) / distributed substrate (g)] * 100;

- waste reduction index (WRI)[20]

WRI = [(W – R) / W] / days of trial (d) * 100

where W = total amount of rearing substrate distributed during the trial (g); R = residue substrate (g);

- efficiency of conversion of digested food (ECD)[20]

ECD = total final biomass (g) / (total feed distributed (g) - residual substrate (g))

where total final biomass = larvae + prepupae; residual substrate = undigested food + excretory products.

Parameters related to waste reduction efficiency (SR, WRI and ECD) were calculated on a fresh matter basis.

Larvae nutritional composition

For each trial, a second set of six replicates per rearing substrate was simultaneously prepared with the aim to rear a sufficient amount of larvae to be analyzed for their proximate composition and fatty acid - FA - profile. Five hundred hand-counted 6-day-old larvae were placed into plastic containers of bigger size (25cm × 33cm × 15cm) than those used for the larvae development and waste reduction efficiency test, following the same relationships between (i) number of larvae / container size surface, and (ii) amount of administered feed / larvae density. The larvae were not handled until the appearance of the first prepupa. Then, each container was checked daily and the identified prepupae were removed. The trial ended when the 30% of the larvae reached the prepupae stage. The remaining larvae were then manually separated from the residual rearing substrate, washed, slightly dried with paper towel, weighed and frozen at -80°C until being freeze-dried.

Chemical analyses of rearing substrates and larvae

Samples of freeze-dried rearing substrates and larvae were ground using a cutting mill (MLI 204; Bühler AG, Uzwil, Switzerland). They were analyzed for DM, ash, CP and EE following AOAC International methods as detailed in Gasco *et al*.[5] For the determination of the CP of whole BSF larvae, in addition to the conventional nitrogen-to-protein (N-factor) conversion factor of 6.25, the more accurate N-factor of 4.67 suggested by Janssen *et al*.[22] was used. Neutral detergent fiber (NDF) was analyzed according to Van Soest *et al*.[23] Acid detergent fiber and acid detergent lignin (ADF and ADL) were determined according to method no. 973·18 of AOAC International.[24] The residual nitrogen in ADF (ADFN) was determined according to method no. 984.13 of AOAC International.[24] Chitin (CHI, g kg^{-1} DM) was estimated as: ash free ADF (g kg^{-1}) – ADFN * N-factor (g kg^{-1}).[9] Gross energy (GE) was determined using an adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany). The FA composition of substrates and larvae was assessed.[25] The results were expressed as g kg^{-1} of total detected fatty acids (TFA) (Table 2).

Statistical analyses

The statistical analysis of data was performed using IBM SPSS Statistics v. 20.0 for Windows. The two trials were considered separately. Larvae weights and lengths were subjected to a Two-Way Mixed ANOVA. The Shapiro-Wilk test was used to verify if the dependent variables were normally distributed for each combination of the groups of within- (test day, considered as a repeated measure) and between- (rearing substrate) subjects factors. The Levene's test was used to verify the homogeneity of variances for each combination of the groups of within- and between-subjects factors. The Mauchly's test was used to verify the assumption of sphericity; if such an assumption was violated, the Greenhouse-Geisser or the Huynh-Feldt correction (in cases of estimates of sphericity lower or higher than 0.75, respectively) was applied to correct the degrees of freedom of the F-distribution. Final larvae weights and lengths (average weight and length of the

leftover 70% larvae after removing the 30% of prepupae) were further subjected to independent samples Student's *t*-tests to assess differences between rearing substrates.

Differences in terms of larvae growth performances, waste reduction efficiency, proximate composition and FA profile between substrates were also assessed using independent-samples Student's *t*-tests.

The Kruskal-Wallis test was used to compare the time needed by the larvae to reach the prepupae stage.

Significance was declared at $P<0.05$.

RESULTS

Growth performances and waste reduction efficiency of the BSF larvae

The effect of the rearing substrate on the development of BSF larvae over time is reported in Figure 1 (Trial 1) and Figure 2 (Trial 2).

In both trials, results from the Two-Way Mixed ANOVA showed that, for larvae development (weight and length), rearing substrate, test day and their interaction were highly significant ($P<0.001$), VEGFRU and BRE performing better than FRU and WIN, respectively.

In Trial 1, no differences were observed for larvae weight (mean ± SD: 0.004±0.0002 g) at the beginning of the trial (day 0; 6 days-old larvae) (Figure 1A). Differences appeared after 4 days of trial, with a higher weight in the VEGFRU larvae (0.055±0.0084 g) compared to the FRU larvae (0.037±0.0058 g). Such trend was maintained at each test day until day 16 (VEGFRU: 0.148±0.0103 g; FRU: 0.120±0.0094 g) when VEGFRU larvae started to enter in the prepupae stage. The final weight of the larvae did not show differences between the two rearing substrates. At the beginning of Trial 1, VEGFRU and FRU larvae showed length values of 5.7±1.32 mm and 5.3±1.17 mm, respectively ($P<0.05$; Figure 1B). VEGFRU larvae continued showing higher length values than FRU larvae until the

last statistical assessment (day 16). VEGFRU and FRU larvae achieved a final length of 17.7±0.46 mm and 17.8±0.51 mm, respectively (P>0.05).

At the beginning of Trial 2, no differences were observed between WIN and BRE for larvae weight (0.007±0.0011 g) (Figure 2A). Remarkable differences were reported after 4 days of trial, with a higher weight in the BRE larvae compared to the WIN larvae (0.092±0.0063 and 0.017±0.0018 g, respectively). The final weight of the larvae (reached after 8 and 26 days of trial for BRE and WIN, respectively) did not show differences between treatments. The mean length of 6-day-old larvae (day 0) was 6.5±1.36 and 6.4±1.24 mm for BRE and WIN, respectively (P>0.05; Figure 2B). After 4 days of trial, differences in larvae length were highlighted, with recorded values of 15.1±1.84 mm (BRE) and 8.7±1.23 mm (WIN).

Dynamic of growth and waste reduction efficiency parameters are reported in Table 1. In Trial 1, VEGFRU larvae showed lower LM and time needed to reach the prepupae stage, as well as higher ECD than FRU larvae. In Trial 2, BRE larvae showed lower LM, time needed to reach the prepupae stage and SR, and contemporarily higher total final biomass, GR, WRI and ECD than WIN larvae.

Proximate and fatty acid compositions of the rearing substrates

The proximate and FA compositions of the rearing substrates are reported in Table 2 and Table 3, respectively.

In Trial 1, VEGFRU showed lower values of DM and NSC and higher contents of ash, CP, NDF and ADF than FRU, while comparable EE and ADL contents were found. In Trial 2, WIN showed higher DM, ash, NDF, ADF, and ADL contents and lower CP and NSC contents than BRE. VEGFRU and FRU showed similar GE values which were lower than those obtained in the second trial for WIN and BRE.

Total FA ranged from 10.04 (FRU) to 82.47 g kg^{-1} DM (BRE). VEGFRU showed higher total polyunsaturated fatty acids (PUFA) and lower total monounsaturated fatty acids (MUFA) than FRU. WIN had higher MUFA and lower SFA when compared to BRE. Linoleic acid (C18:2 n6) was the most abundant FA in all substrates.

Proximate and fatty acid compositions of the BSF larvae

The proximate and FA compositions of the BSF larvae are reported in Table 4 and Table 5, respectively.

Concerning Trial 1, ash, CP and ADF values in the VEGFRU larvae were higher than those in FRU larvae. Conversely, the FRU larvae showed higher DM, EE and NDF contents than the VEGFRU larvae. In Trial 2, the WIN larvae showed lower DM and CP contents when compared to the BRE larvae, while all the other parameters showed an opposite trend.

Considering the FA composition of the larvae, FRU larvae showed higher TFA than VEGFRU larvae. On the contrary, in Trial 2 similar TFA contents were observed for BRE and WIN larvae. Significant differences between treatments were observed in both trials for almost all considered FA groups and individual FA. PUFA were higher in VEGFRU and BRE larvae when compared to FRU and WIN larvae, respectively, while an opposite trend was observed for SFA. The most represented individual FA in BSF larvae from all treatments was C12:0, which showed higher amounts in FRU and WIN when compared to VEGFRU and BRE, respectively. C18:1 *c*9 and C18:2 n6 were the most represented unsaturated FA in all treatments.

DISCUSSION AND CONCLUSIONS

Our study investigated, through 2 trials, the effects of different rearing substrates on development, waste reduction efficiency, and nutritional composition of BSF larvae.

VEGFRU and BRE larvae showed higher weights after 4 days from the beginning of the trial, had lower mortality and needed less time to reach the prepupae stage than FRU and WIN larvae, respectively. Such results were obtained in spite of comparable GE values found in Trial 1 for VEGFRU and FRU and in Trial 2 for WIN and BRE substrates, and can be at least partly ascribed to the higher CP and

moisture contents of VEGFRU and BRE, confirming the results obtained by other authors.[26,27]

The need for high dietary moisture content could be ascribed to the morphology of the mouthparts of BSF larvae, which resembles the characteristics of scavenger insects.[28,29] This kind of macerating mouth apparatus allows BSF larvae to scrape off the food from the feeding surface. By softening the feed solids, increased dietary moisture content makes easier for the larvae to feed.[30]

The results obtained in our trials could be also reflective of possible differences between rearing substrates in terms of the content of nutrients other than CP (e.g., ether extract, structural and non-structural carbohydrates, amino acids) and/or in terms of nutrient digestibility. In both trials, the EE content of substrates (<90 g kg^{-1} DM) was far below the 200-260 g kg^{-1} found by Nguyen *et al.*[12,13] to have detrimental effects for the survival of BSF larvae and adults. BRE larvae showed very good performances despite the high structural carbohydrates content of the relative rearing substrate (NDF: 447 g kg^{-1} DM; ADF: 225 g kg^{-1} DM). Such a result clearly demonstrates that BSF larvae are also able to efficiently bioconvert wastes and by-products characterized by high fiber content, thanks to the presence, in the digestive tract of the insect, of intestinal bacteria able to degrade cellulose.[31] The amino acid composition of the rearing substrates was not analyzed in our trials, and little literature is available concerning the effects of dietary amino acids on development and nutritional composition of BSF larvae.[32,33] Studying the nutritional composition of BSF prepupae reared on different organic waste substrates, Spranghers *et al.*[32] showed that the amino acid content of the prepupae had narrow ranges, particularly when compared to the noticeable differences found in the amino acid composition of the rearing substrates. Concerning nutrient digestibility, to the best of our knowledge no studies are currently available. Further studies are necessary to deepen these aspects for the optimization of BSF feeding and nutrition.

In Trial 2 the differences in larvae growth performances between treatments were very pronounced. We may speculate that the GE of the WIN substrate was not fully available for the larvae. The methodology used to grind the WIN substrate could have influenced the availability of the oil present inside the grape seeds. Indeed, a 3-mm grinder was used, and this size could not have completely milled the seeds. Moreover, the WIN substrate could have contained substances unsuitable for the BSF larvae development. Indeed, winery by-products usually contain high levels of polyphenols.[34] It is known that plants use polyphenolic compounds to protect themselves from herbivore insect attacks.[35] Some studies also showed how the grape seeds can accumulate high doses of pesticides and insecticides used in wine grapevines management.[35,36]

Hard *et al*.[37] reported that larvae rearing density affects competition for food, low densities usually leading to highest larvae weights. This was also reflected in our trial, as no (Trial 1) or slight (Trial 2) differences were observed for the total final biomass despite the differences found in LM between treatments. The observed LM for VEGFRU was lower than that reported by Nguyen *et al*.[13] using a vegetable and fruit rearing substrate.

In both trials, the differences highlighted in terms of LM and ECD were closely connected, and treatments leading to lower mortality allowed obtaining the best performances in terms of ECD. BRE larvae reduced a lesser quantity of substrate compared to WIN larvae; nevertheless, the WRI was higher in the BRE larvae as they took less time to reach the prepupae stage, which is also confirmed by the higher GR results. The SR was particularly high (above 65%) in Trial 1, showing the great potential of BSF larvae in the degradation of vegetable and fruit wastes.[12,13] Overall, the BRE larvae showed the best ECD combined with the absolute highest total final biomass production and the shortest developmental period.

The time needed by the larvae to reach the prepupae stage seemed to influence their chitin content. Such results agree with the findings of Diener *et al*.[11] who

reported how small larvae grown in 42 days showed a chitin level higher than heavy larvae grown in 16 days.

In both trials, substrates containing the highest CP and moisture contents (VEGFRU and BRE) allowed obtaining BSF larvae with the highest CP level, which is consistent with the results obtained by other authors.[26,27] Consistently with the findings of Janssen *et al.*,[22] the use of the conventional N-factor of 6.25 led to a CP overestimation of about 25%.

Despite comparable EE values of the rearing substrates, FRU larvae showed higher EE content than VEGFRU larvae, probably as a consequence of the higher NSC level of FRU.[38] Insects have the ability to convert carbohydrates into lipids.[32,39] Insects store lipids for two reasons. Firstly, as energy reserve for the adult stage.[14] Secondly because, as insect body presents an open blood system and a high surface compared to volume and the combination of these two factors could be a problem for the loss of water and the drying out process, lipids allow them to avoid transpiration and store non-imbibed water.[40] However, the influence of the NSC content of substrates on the EE content of BSF larvae should be further investigated as higher NSC in BRE substrate did not lead to higher EE content in BSF larvae in Trial 2.

The FA composition of the rearing substrates did not directly affect the larvae FA composition, which was also influenced by carbohydrates (starch and sugars), confirming other researches.[26,32] Being of vegetable origin, all rearing substrates had PUFA as the most abundant FA group. Notwithstanding, as typically observed for Diptera, the BSF larvae FA profile was dominated by SFA, mainly C12:0 (which showed the absolute highest values among individual detected FA), C14:0, C16:0 and C18:0.[32,41] The high presence of SFA in insects is connected with cold-adaptation.[42] Indeed, larvae from some species showed a SFA decrease from summer to autumn while PUFA increased highlighting a correlation between the change in FA composition and the temperatures due to seasonal change.[42,43,44] BSF is a sub-tropical species growing with high temperatures (27-32°C) and the difficult

adaptation to low temperatures was demonstrated by the lowest BSF survival rate at about 16°C.[45] We can argue that the high SFA presence could be ascribed to BSF adaptation to the sub-tropical climate. In particular the high content of lauric acid (melting point: 43.2°C) could preserve BSF larvae from lipid oxidation and allow them to survive at temperatures above 40°C.[41] Consistent with other findings,[7,15,32] C18:1 *c*9 was the main represented MUFA in the larvae, while C18:2 n6 and C18:3 n3 were the main represented PUFA n6 and PUFA n3, respectively. The low quantity of recovered n3 PUFA in the larvae could represent a problem if insect meals are intended to be used for animal feed. Indeed, researches highlighted a decrease in nutritional product quality with the inclusion of insect meals in animal diets especially when full-fat meals are used.[5,6,46] Nevertheless, BSF larvae can be enriched in n3-PUFA through the substrate.[33,47] Authors [10,48] reported that C12:0 is a good inhibitor of bacteria strains and could be of great interest in the reduction of the use of antibiotics in animal feeding.[10,49,50] In this context, BSF larvae reared on organic wastes resulted very interesting with up to 574 g kg^{-1} TFA of lauric acid.

Especially in Southern Europe, the large availability of vegetable and fruit wastes (mainly from markets and supermarkets) may allow the development of a BSF larvae mass production, enabling as well to obtain economic and environmental benefits from the sustainable management of organic wastes. Regarding the considered agro-industrial by-products, the use of winery by-products as rearing substrate for BSF larvae could be conditioned, both from a technological and economical point of view, by the need of preliminarily processing to remove or reduce polyphenols, pesticides and insecticides contents, which could exert a negative influence on the growth performance of the larvae. Remarkable positive results were obtained in terms of overall development time, growth performances and nutritional composition for the larvae reared on brewery by-products, which should therefore be considered promising rearing substrates. However, as brewery by-products are characterized by a more limited availability than vegetables, fruits

or winery by-products, it could be advisable to use BRE at low dietary inclusion levels with the purpose of increasing BSF larvae performances.

Overall, our results show that the performance and chemical composition of BSF larvae are largely affected by the chemical composition of the provided substrate. This clearly demonstrates that insects, like farm animals, have nutritional requirements which have to be met for optimal performance.

The performances obtained in our bench top trials may vary when transferred to an industrial scale. For instance, the large volumes of waste used as well as the high larvae concentration could result in environmental oxygen depletion and heat production.[51] Attention should then be placed to the airflow to guarantee appropriate rearing conditions. In addition, to optimize land use, insect breeding should exploit the verticality of the breeding structure. However, this can lead to the stratification of temperature and particular attention must be given to an adequate air circulation to guarantee a homogeneous temperature in all parts of the building. At industrial level, the production system would also require a constant supply of substrates, possibly with a fairly constant chemical composition, as to obtain BSF larvae with relatively constant nutrient profile.

Future studies should be designed to assess the nutritional requirements of BSF larvae and to evaluate other agro-industrial by-products, as well as the effect of mixing different organic wastes and agro-industrial by-products, to obtain optimal BSF larvae performances in terms of development, waste reduction efficiency and nutritional composition.

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Table 1: Dynamic of growth and waste reduction efficiency (on a fresh matter basis) of black soldier fly larvae reared on organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector (mean ± SD; n = 6).

	Trial 1 (organic wastes)			Trial 2 (agro-industrial by-products)		
	VEGFRU	FRU	*P*	WIN	BRE	*P*
Larvae mortality (%)	11.2±4.35	19.3±5.24	0.015	24.8±10.53	9.5±5.68	0.011
Total final biomass (g)	10.42±0.648	10.92±2.057	0.584	9.90±0.785	11.32±0.864	0.014
Time needed to reach prepupae stage (days of trial)	20.2±1.33	22.0±0.89	0.031	22.2±0.98	8.0±0.01	0.003
Growth rate (g d^{-1})	0.006±0.0018	0.007±0.0007	0.451	0.006±0.0009	0.014±0.0009	0.000
Substrate reduction (%)	65.2±5.54	70.8±8.39	0.129	53.0±5.28	42.5±8.41	0.027
Waste reduction index (g d^{-1})	3.2±0.26	3.2±0.41	0.952	2.4±0.32	5.3±1.05	0.000
Efficiency of conversion of digested food	0.07±0.009	0.05±0.011	0.004	0.06±0.002	0.14±0.034	0.000

VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

Table 2: Proximate composition (g kg^{-1} dry matter, unless otherwise stated) of organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector and used as rearing substrates by black soldier fly larvae.

	Trial 1 (organic wastes)		Trial 2 (agro-industrial by-products)	
	VEGFRU	FRU	WIN	BRE
Dry matter (g kg^{-1})	82.7	131.9	358.3	232.1
Ash	91.1	30.4	103.0	39.8
Crude protein	119.9	46.0	117.4	200.5
Ether extract	26.0	27.8	79.0	86.7
Neutral detergent fiber	178.0	139.3	566.4	447.1
Acid detergent fiber	110.5	91.1	462.4	225.3
Acid detergent lignin	12.9	13.1	323.5	62.1
Non-structural carbohydrates*	585.0	756.5	134.2	225.9
Gross energy (MJ kg^{-1} DM)	15.1	15.6	19.5	19.4

VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

*Calculated as: 1000 – (crude protein + ether extract + ash + neutral detergent fiber).

Table 3: Fatty acid composition (g kg^{-1} total fatty acids, unless otherwise stated) of organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector and used as rearing substrates by black soldier fly larvae.

	Trial 1 (organic wastes)		Trial 2 (agro-industrial by-products)	
	VEGFRU	FRU	WIN	BRE
Total fatty acids (g kg^{-1} dry matter)	20.91	10.04	73.57	82.47
C12:0	0.73	3.52	0.93	0.80
C14:0	8.85	8.44	1.99	3.22
C16:0	184.90	192.71	100.49	252.48
C16:1 *c*9	5.89	5.96	4.43	1.75
C18:0	26.14	43.36	50.24	15.42
C18:1 *c*9	65.91	208.61	185.09	103.27
C18:1 *c*11	20.85	29.62	8.51	7.46
C18:2 n6	575.23	333.38	630.32	554.90
C18:3 n3	111.50	174.40	18.00	60.70
Saturated fatty acids	220.62	248.03	153.65	271.92
Monounsaturated fatty acids	92.65	244.19	198.03	112.48
Polyunsaturated fatty acids	686.73	507.78	648.32	615.60

VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

Table 4: Proximate composition (g kg^{-1} dry matter, unless otherwise stated) of black soldier fly larvae reared on organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector (mean ± SD; n = 6).

	Trial 1 (organic wastes)			Trial 2 (agro-industrial by-products)		
	VEGFRU	FRU	*P*	WIN	BRE	*P*
Dry matter (g kg^{-1})	219.6±10.22	282.9±6.57	0.000	265.4±5.93	290.8±6.96	0.000
Ash	129.8±6.50	72.2±2.22	0.000	145.7±6.67	73.0±1.89	0.000
Crude protein[1]	418.8±13.24	307.5±10.29	0.000	344.3±7.63	529.6±5.27	0.000
Crude protein[2]	312.9±9.89	229.7±7.69	0.000	257.3±5.70	395.7±3.94	0.000
Ether extract	262.8±18.01	407.0±18.83	0.000	322.2±19.60	298.7±6.49	0.031
Neutral detergent fiber	170.9±16.49	197.9±13.48	0.011	177.3±13.08	87.0±9.89	0.000
Acid detergent fiber	113.1±20.09	93.4±3.55	0.014	98.5±10.16	64.8±9.17	0.000
Acid detergent lignin	14.9±7.75	8.9±2.47	0.104	44.8±17.80	8.3±9.35	0.001
Chitin[3]	62.4±19.63	56.0±3.96	0.453	52.9±9.25	14.2±6.06	0.000
Chitin[4]	75.2±19.7	65.5±3.53	0.283	64.5±9.48	27.0±6.59	0.000

VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

[1] Obtained using the nitrogen-to-protein conversion factor of 6.25.

[2] Obtained using the nitrogen-to-protein conversion factor of 4.67.

[3] Calculated using the nitrogen-to-protein conversion factor of 6.25.

[4] Obtained using the nitrogen-to-protein conversion factor of 4.67.

Table 5: Fatty acid composition (g kg^{-1} total fatty acids, unless otherwise stated) of black soldier fly larvae reared on organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector (mean ± SD; n = 6).

	Trial 1 (organic wastes)			Trial 2 (agro-industrial by-products)		
	VEGFRU	FRU	*P*	WIN	BRE	*P*
TFA (g kg^{-1} dry matter)	253.02±18.512	398.40±18.547	0.000	287.41±16.973	282.93±6.936	0.563
C12:0	520.61±17.505	574.32±11.060	0.000	346.91±16.840	323.73±9.277	0.014
C14:0	103.55±3.303	96.39±3.471	0.004	65.54±4.283	66.49±2.687	0.654
C16:0	138.95±7.338	130.57±3.846	0.040	189.36±7.434	204.15±5.772	0.003
C16:1 *c*9	33.57±3.606	37.45±0.956	0.046	60.63±4.718	29.45±2.639	0.000
C18:0	25.90±1.693	17.51±0.539	0.000	28.32±2.139	18.07±0.599	0.000
C18:1 *c*9	85.37±4.075	93.19±2.086	0.002	124.59±4.280	92.23±2.414	0.004
C18:1 *c*11	4.31±0.381	2.79±0.157	0.000	4.46±0.261	5.75±1.155	0.040
C18:2 n6	70.41±7.408	40.70±1.534	0.000	175.76±14.935	235.47±6.593	0.000
C18:3 n3	17.31±1.370	7.06±0.729	0.000	4.44±0.392	24.65±0.504	0.000
SFA	789.02±10.854	818.81±4.632	0.000	630.13±16.745	612.45±8.784	0.045
MUFA	123.26±6.829	133.44±2.773	0.013	189.68±6.220	127.43±5.354	0.000
PUFA	87.72±7.333	47.75±2.083	0.000	180.19±15.244	260.12±6.843	0.000

VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

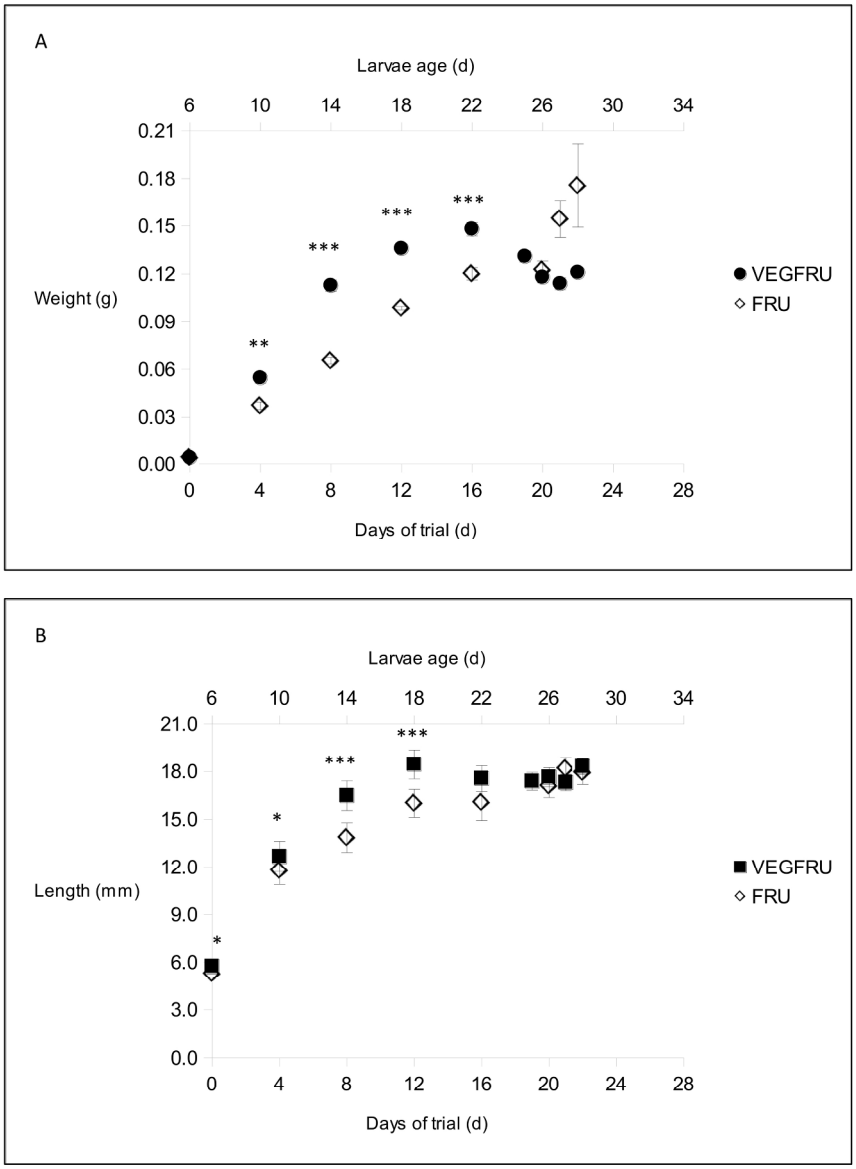

Figure 1. Trial 1: Development (A: weight; B: length) of black soldier fly larvae reared on organic wastes (VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste) generated by the Italian food sector. P-value: *P<0.05, **P<0.01, ***P<0.001. Error bars represent the standard error of the mean.

106x140mm (600 x 600 DPI)

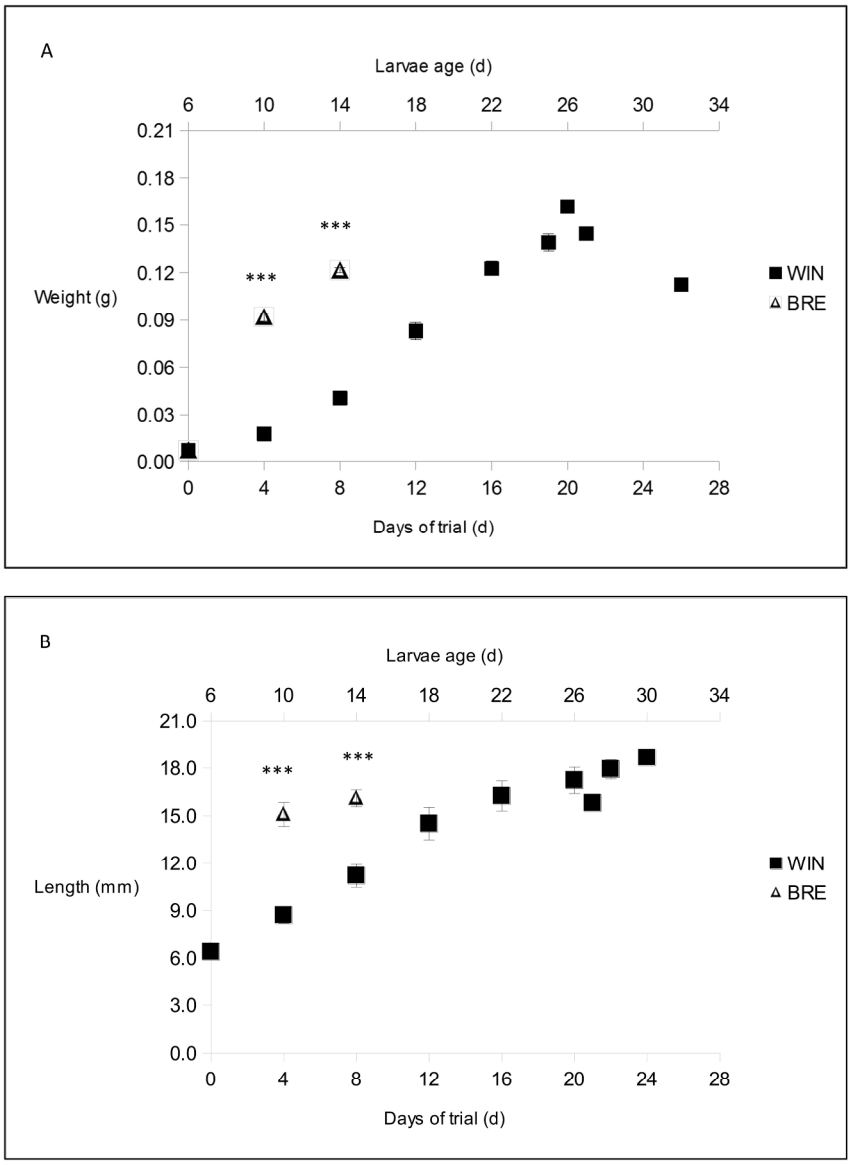

Figure 2. Trial 2: Development (A: weight; B: length) of black soldier fly larvae reared on agro-industrial by-products (WIN: winery by-product; BRE: brewery by-product) generated by the Italian food sector. P-value: *P<0.05, **P<0.01, ***P<0.001. Error bars represent the standard error of the mean.

107x140mm (600 x 600 DPI)

Figure 1. Trial 1: Development (A: weight; B: length) of black soldier fly larvae reared on organic wastes (VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste) generated by the Italian food sector. *P*-value: *P<0.05, **P<0.01, ***P<0.001. Error bars represent the standard error of the mean.

Figure 2. Trial 2: Development (A: weight; B: length) of black soldier fly larvae reared on agro-industrial by-products (WIN: winery by-product; BRE: brewery by-product) generated by the Italian food sector. *P*-value: *P<0.05, **P<0.01, ***P<0.001. Error bars represent the standard error of the mean.